International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.1, pp 148-152, Jan-Mar 2011

Evaluation of antioxidant and antimicrobial activities of the selected green leafy vegetables

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Abstract: The aim of the present study is to evaluate the antioxidant and antimicrobial activity of the selected green leafy vegetables such as *Amaranthus tristis, Centella asiatica, Hibiscus sabdariffa, Moringa oleifera, Sesbania grandiflora* and *Solanum trilobatum.* The antioxidant screening was done by DPPH free radical scavenging assay using aqueous and ethanolic extracts. The antimicrobial activity of the plant extracts were done against *E.coli*, *Staphyloccoccus aureus* and *Klebsiella pneumonia*. Present study showed strong antioxidant activity of ethanolic extracts of *Hibiscus sabdariffa, Centella asiatica* and the aqueous extract of *M.olifera.* Antimicrobial activity was observed from the ethanolic extracts of only three samples.

Keywords: Leafy vegetables, antioxidants, phytochemicals and antimicrobial activity.

Introduction

In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes [1, 2]. Free radicals are highly reactive compounds, they are chemical species associated with an odd or unpaired electron and can be formed when oxygen interacts with certain molecules. They are neutral, short lived, unstable and highly reactive to pair with the odd electron and finally achieve stable configuration. Once formed these highly reactive radicals can start a chain reaction they are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cells may function poorly or die if this occurs[3].

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules and thereby reduces the risk of cancer and other diseases. The major action of antioxidants in cells is to prevent damage due to the action of reactive oxygen species. Studies have suggested that antioxidant supplements have benefits for health. All living organisms contain complex systems of antioxidant enzymes and chemicals, some to combat oxidative damage to cellular components and others to regulate and sustain natural cellular processes such as oxidative phosphorylation and the formation of disulfide bonds [4]. All cells contain complex systems of antioxidants to prevent chemical damage to the cells components by oxidation.

Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects.

The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials. The possible toxicity of synthetic antioxidants has resulted in decreased use of these

compounds in foods for human consumption. As a consequence of this and due to the appeal of natural products to consumers, numerous studies have been carried out in order to identify naturally occurring compounds which possess antioxidant activities such as phenolic photochemical [5].

Nearly one thousand species of plants with edible leaves are known. Leafy vegetables most often come from short lived herbaceous plants such as lettuce and spinach. Woody plants whose leaves can also be eaten as leafy vegetables. For the present study the following green leafy vegetable viz. *Amaranthus tricolor* L., *Centella asiatica* L., *Hibiscus sabdariffa* L., *Moringa oleifera* Lam., *Sesbania grandiflora* (L.) Poir. and *Solanum trilobatum* L. were investigated for the antioxidant and antimicrobial activities. The presence of carotenes, phenolic compounds such as flavanoids, coumarins, alkaloids, etc. were also investigated.

Materials and Methods

Plant Material

Green leafy vegetables used in the study were collected from Koyambedu market, Chennai of Tamil Nadu, India, the collected materials were authenticated by Dr.C.Livingstone, Head of the Department of Botany, Madras Christian College, Chennai and voucher specimens were deposited in the herbarium of the institute.

Preparation of Extracts

Healthy plants were collected, shade dried and powdered. Ten gram of the respective powder was mixed separately with 100ml of distilled water and ethanol for aqueous and ethanol extraction respectively. These mixtures were incubated for 24 hours with occasional shaking and filtered with Whatman filter paper to obtain filtrate which was further evaporated to obtain the extract. For the experiment to be performed stock solutions of aqueous and ethanol extracts of 1mg/ml was prepared.

Phytochemical Screening of the Extracts

Phytochemical screening was performed using standard procedures as described by Sofowora [6] and Trease & Evans [7].

Test for alkaloids: Two tests were performed using Wagner's reagent and the other using Hager's reagent respectively.

- To the aliquot of diluted extracts Wagner's reagent was added. Reddish brown colour precipitation indicates the presence of alkaloid.
- To the aliquot of diluted extracts Hager's reagent was added. Yellow colour precipitation indicated the presence of alkaloid.

Test for flavanoids: The various extracts were treated with amyl alcohol and sodium acetate. After 5mins the reaction mixture was treated with ferric chloride. The appearance of pink or red color confirms the presence of flavonoids.

Test for tannins: To the aliquot of diluted extracts, 2ml of 10% lead acetate was added. White colour precipitation indicated the presence of tannins.

Test for coumarins: To the aliquot of diluted extracts, 1ml of 2N NaOH was added. Formation of yellow colour indicated the presence of coumarins. Further 1ml of 5N HCl was added. A colourless solution was formed at the upper layer and it confirmed the presence of coumarins.

Test for Sterols: This test is known as Sakowski test. The diluted extract was dissolved in 1ml of chloroform; 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. The red colour produced indicates the presence of sterols

Test for Amino Acids: To an aliquot of diluted extracts, 2ml of ninhydrin solution was added. Violet colour formation indicated the presence of amino acid

Test for proteins: To 1ml of diluted extract, 1ml of 5% $CuSO_4$ and 1% NaOH solution was added. Deep blue colour confirmed the presence of proteins.

Estimation of Carotenes in Green Leafy Vegetables

Total carotene content was estimated by the standard method prescribed by Arnon[8]. One gram of fresh sample was weighed and made into a paste with 10 ml of distilled water. From the above sample 0.5 ml extract was mixed with 4.5 ml 80% acetone and the OD value was observed at varying wavelengths like 490nm, 638nm, 645nm and 663nm. The amount of carotene was calculated using the following formula

= (O.D 490) - (0.114*O.D 663)-(0.638*O.D 645)

DPPH Free Radical Scavenging Assay

The free radical scavenging activities of the plant extracts against 2, 2 Diphenyl-1- Picryl Hydrazyl reduced were determined. Varying concentrations of the herbal extract were prepared from the stock solution. The reaction consisted of 1ml of 0.1mM DPPH in ethanol, 1ml of 0.05M tris HCl buffer, 1ml ethanol and 0.5ml of herbal extract. The tubes were kept in the dark and the absorbance was measured at 520nm. The decrease in absorbance at 520 nm was continuously recorded in a spectrophotometer for 10 min. the scavenging effect, expressed as the decrease of absorbance at 520 nm was observed and the percentage of DPPH radical-scavenging ability

(SA) of the various extracts were calculated using the following formula.

$$\% \text{ inhibition} = \frac{A_0 - A_{10}}{A_0} * 100$$

Where A_0 = absorbance at 0 min; A_{10} = absorbance at 10 min.

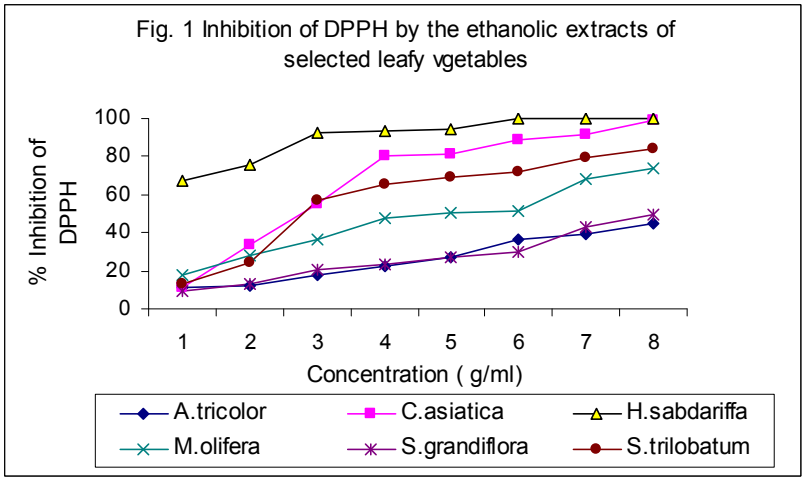
Screening for Antimicrobial Activity

The antimicrobial activities of the crude aqueous and ethanolic extracts of the plant samples were evaluated by antibiotic well assay method [9]. The solidified LB agar plates were inoculated with 100 µl of *E.coli* (MTCC-443), *Klebsiella pneumonia* (MTCC-109) and *Staphylococcus aureus* (MTCC-96) by swabbing. The wells were prepared on the agar plate already seeded with cultures (10^6 cfu/ml) with the help of a cork borer (10mm dia.). The wells were loaded with 50, 100,150 and 200 µg/ml volume of the extracts and 50 µl of ampicillin was used as a control. The plates were incubated at 37° C overnight. For each extract three replicate trials were conducted against each organism.

Table.1 Phytochemical constituents of selected green leafy vegetables.

Reaction	*A.tricolor*	*C. asiatica*	*H. sabdariffa*	*M. oleifera*	*S.grandiflora*	*S.trilobatum*
Alkaloid	+	+	+	+	+	+
Flavanoid	+	+	+	-	+	+
Tanin	-	+	-	-	+	+
Couramins	+	+	+	+	+	+
Sterols	+	+	+	-	+	+
Amino acids	+	+	+	+	+	+
Proteins	+	+	+	+	+	+

+ sign indicate response and – sign indicates no response.



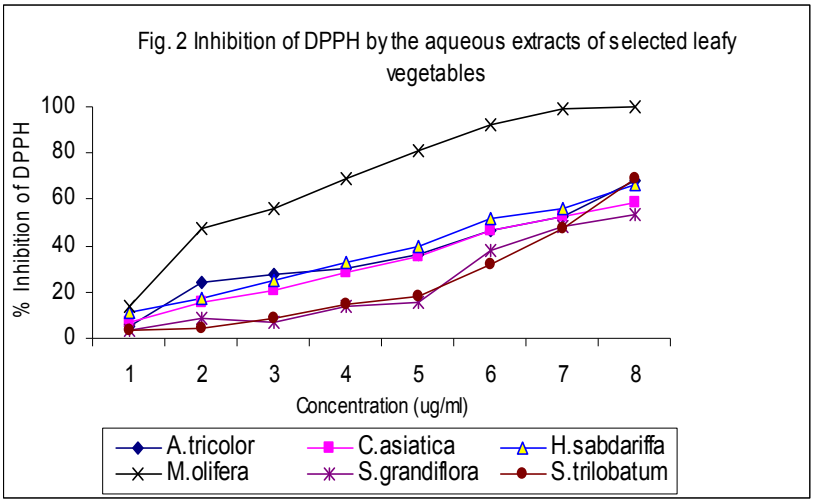


Table.2 Antimicrobial activity of selected green leafy vegetables.

Extracts		E.coli	K.pneumoniae	S.aureus
Ampicillin(50µg/ml)		+	+	+
A.tricolor (200µg/ml)	Aqueous	-	-	-
	Ethanolic	-	-	-
C. asiatica (200µg/ml)	Aqueous	-	-	-
	Ethanolic	-	-	-
H. sabdariffa (200µg/ml)	Aqueous	-	-	-
	Ethanolic	-	-	-
M. oleifera (200µg/ml)	Aqueous	-	-	-
	Ethanolic	+	+	+
S.grandiflora (200µg/ml)	Aqueous	-	-	-
	Ethanolic	+	-	-
S.trilobatum (200µg/ml)	Aqueous	-	+	+
	Ethanolic			

+ sign indicate activity and – sign indicates no activity.

Results

Phytochemical Screening of the Extracts

Phytochemical screening of all the plants tested revealed the presence of coumarins, amino acids and proteins (Table-1). All the samples showed positive result for alkaloid, except *M.olifera* all other samples showed positive results for flavanoid and sterols. S.trilobatum, *C.asiatica* and *S.grandiflora* tested positive for the presence of tannins.

DPPH Free Radical Scavenging Assay

All the evaluated samples showed moderate to little free radical scavenging activities. The ethanolic extract of *H. sabdariffa* showed 98% free radical inhibition at 250 µg/ml concentration followed by *C.asiatica* which expressed 100% inhibition at 400 µg/ml concentration (Fig.1). Extracts from other species also showed comparable amount of DPPH inhibition activities. While in the case of Ascorbic acid (Vitamin C) 100% inhibition was noticed at 7 µg/ml concentration. But among aqueous extracts only *M.olifera* showed strong free radical inhibition of 100% inhibition at 400 ug/ml concentration (Fig.2). Comparative analysis indicated that *H. sabdariffa* is about 30 times less potent than the standard (ascorbic acid) and reasonable amount of free radical inhibition activity was also observed from *C.asiatica and M.olifera.*

Estimation of Carotenes in Green Leafy Vegetables

Among the various plant extracts tried, *H. sabdariffa* was found to have maximum amount of carotene (0.47 g/l) followed by *A.tristis*(0.44 g/l) and appreciable amount of carotene was also observed from other species (Fig.3).

Screening for Antimicrobial Activity

Antimicrobial activity was not exhibited by the crude aqueous extracts of these selected plant samples. The crude ethanolic extract of *Solanum trilobatum* inhibited *S.areus* and *K.pneumonia.*

M.olifera exhibited antimicrobial activity against *E.coli*, *S.areus* and *K.pneumonia* and *S.grandiflora* against *E.coli* (Table.2). *T*he antimicrobial activity of ampicillin standard also showed inhibitory effect on E.coli, K.pneumoniae and S.aureus.

Discussion

Phytochemical screening of the plant extracts revealed some difference between the plants. S.trilobatum, *C.asiatica* and *S.grandiflora* extracts showed positive results for all the classes of chemicals.

Greater inhibition of free radicals in *H. sabdariffa, C.asiatica* and *M.olifera* may be due to the presence of high amount of alkaloids and flavanoids. The presence of flavanoids and tannins in all the plants are likely to be responsible for the free radical scavenging effects observed. Flavanoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as a primary antioxidant or free radical scavenger. Earlier Anita and Jayashree (1999) have also reported that many green leafy vegetables of Indian regions are rich sources of antioxidants.

The lack of antimicrobial activity in aqueous extracts and some ethanolic extracts may be due to the absence of antimicrobial components in these extracts. It was also reported [10] that leaf extracts contain less antimicrobial activity compared to stem and other parts, due to the interference of pigments and phenolics with the antimicrobial activity of these extracts. From this, it is obvious that the lack of antimicrobial activity in these extracts may be due to the presence of pigments like carotene and phenolics in these plants. Since the selected leafy vegetable samples are rich in carotene and varying levels of antioxidants, inclusion of these in the diet is likely to reduce oxidative stress.

References

1. Etkin N.L. 1996. Medicinal cuisines: diet and ethnopharmacology. Int.J.Pharmacognosy 34:313-326.
2. Pieroni A. 2000. Medicinal plants and food medicines in the folk traditions of the upper Lucca Province, Italy. J.Ethnopharmacol. 70:235-237.
3. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci* 1993; 90:7915.
4. Wang JY, Wen LL, Huang YN, Chen YT, Ku MC. (2006). Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. Curr Pharm Des 12(27):3521-33.
5. Nantz MP, Rowe CA, Nieves C Jr, Percival SS. (2006). Immunity and antioxidant capacity in humans is enhanced by consumption of a dried, encapsulated fruit and vegetable juice concentrate. J Nutr 136(10):2606-10.
6. Sofowora A. 1993. Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan. pp 150.
7. Trease G.E and Evans W.C. 1989. Pharmacognosy. 13th edn. Bailliere Tindall, London. pp 176-180.
8. Arnon D. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15.
9. Perez C, Pauli M and Bazevque P. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis. 1990;15:113-115
10. Doughari J.H. 2006. Antimicrobial activity of *Tamarindus indica* Linn. Trop.J.Pharm.Res. (2): 597-603.

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